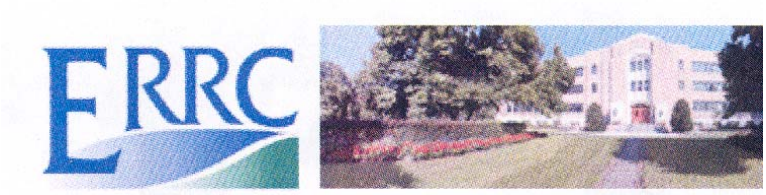

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Title: Inactivation of *Escherichia Coli* O157:H7 and *Salmonella* by Gamma Irradiation of Alfalfa Seed Intended for Production of Food Sprouts

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Inactivation of *Escherichia coli* O157:H7 and *Salmonella* by Gamma Irradiation of Alfalfa Seed Intended for Production of Food Sprouts†

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ABSTRACT

Inonizing irradiation was determined to be a suitable method for the inactivation of *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seed to be used in the production of food sprouts. The radiation *D* (dose resulting in a 90% reduction of viable CFU) values for the inactivation of *Salmonella* and *E. coli* O157:H7 on alfalfa seeds were higher than the *D*-values for their inactivation on meat or poultry. The average *D*-value for the inactivation of *Salmonella* on alfalfa seeds was 0.97 ± 0.03 kGy; the *D*-values for cocktails of meat isolates and for vegetable-associated isolates were not significantly different. The *D*-values for nonoutbreak and outbreak isolates of *E. coli* O157:H7 on alfalfa seeds were 0.55 ± 0.01 and 0.60 ± 0.01 kGy, respectively. It was determined that the relatively high *D*-values were not due to the low moisture content or the low water activity of the seed. The *D*-values for *Salmonella* on alfalfa seeds from two different sources did not differ significantly, even though there were significant differences in seed size and water activity. The increased moisture content of the seed after artificial inoculation did not significantly alter the *D*-value for the inactivation of *Salmonella.* The results of this study demonstrate that 3.3- and 2-log inactivations can be achieved with a 2-kGy dose of ionizing radiation, which will permit satisfactory commercial yields of sprouts from alfalfa seed contaminated with *E. coli* O157:H7 and *Salmonella,* respectively.

The International Sprout Growers Association estimates that $250,000,000 worth of food sprouts are grown in the United States annually by approximately 475 growers. Several types of seeds are sprouted to provide fresh sprouts for the consumer. Such products include alfalfa, clover, sunflower, broccoli, mustard, radish, garlic, dill, pumpkin, mung bean, kidney bean, pinto bean, navy bean, and soy bean sprouts. Sprouts are often consumed raw in the United States, which means that any pathogenic contaminant either in or on the raw sprout may put the consumer at risk. Unfortunately, the consumption of raw vegetable sprouts has been linked to several outbreaks of foodborne illness *(15).* In 1995, one lot of alfalfa seeds was associated with a major international outbreak of salmonellosis in Denmark, Canada, and the United States *(1, 26).* The consumption of radish sprouts contaminated with *Escherichia coli* O157:H7 was linked to nearly 6,000 cases of illness in Sakai City, Japan, in 1996 *(16).* In 1998, the state of California imposed a health restriction with regard to the consumption of raw sprouts after three outbreaks attributable to sprouts contaminated with *Salmonella* and *E. coli* O157:H7 occurred. In each of these cases, the seeds were the suspected carrier of the pathogen. It is apparent that the warm and moist conditions used to grow sprouts are also ideal for the propagation of foodborne pathogens should they be present on the seeds. In 1999, outbreaks of *Salmonella* Mbandaka associated with the ingestion of alfalfa sprouts linked to contaminated seed from southern California occurred in Idaho, Oregon, and Washington *(12).* On 9 July 1999, the U.S. Food and Drug Administration (FDA) issued the following warning: "Because of reports of increasing numbers of illnesses associated with consumption of raw sprouts, the FDA is advising all persons to be aware of the risks associated with eating raw sprouts (e.g., alfalfa, clover, radish). Outbreaks have included persons of both genders and all age categories. Those persons who wish to reduce the risk of foodborne illness from sprouts are advised not to eat raw sprouts."

Treatments with disinfectants such as hypochlorite have not completely eliminated these pathogens from seeds, nor have they been very effective in decontaminating sprouts *(6, 22).* The objective of this study was to determine whether treating seeds intended for the production of food sprouts with ionizing radiation was a feasible method for decontaminating these seeds.

MATERIALS AND METHODS

Alfalfa seed. Standard commercial alfalfa (*Medicago sativa: Leguminosae*) seeds intended for the production of food sprouts were provided by Caudill Seed Co., Louisville, Ky., and International Specialty Supply, Cookeville, Tenn. The seed supplied by Caudill Seed Co. was described as "Aust. Alfalfa: 99.64% pure

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† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

seed, 0.36% inert matter, 83% germination, 14% abnormal sprouts, 1% dead seed, and 2% hard seed." The seed supplied by International Specialty Supply was described as "Alfalfa, common, buffed." Each batch of seed was handled independently. The physical characteristics of each of these batches of seed were determined as follows.

To determine seed mass, 10 seeds were selected randomly from approximately 500 g of seeds and weighed. This procedure was repeated 10 times.

For irradiation, bulk density is defined as the mass per unit volume that will be placed in the irradiator. It differs from density in that it includes the volume between the seeds in the container. The bulk density of the seeds was determined by weighing three separate 100-ml amounts of the seed.

The moisture content of triplicate 5-g samples of seed was determined by microwave CEM methods (CEM Corp., Matthews, NC) *(5)*.

Water activity (a_w) was determined by dew point analysis *(5)* of triplicate 5-g samples with an Aqualab CX-2T A_w Meter (Decagon Devices, Inc., Pullman, Wash.). Standardization of the instrument was carried out with 6 M NaCl (a_w = 0.760).

Seeds used for determination of radiation *D*-values. Seeds intended for use in the determination of radiation *D* (dose resulting in a 90% reduction of viable CFU) values were vacuum packaged in 50-g amounts in Stomacher 400 polyethylene bags (Tekmar Co., Cincinnati, Ohio). After the seeds were packaged, they were sterilized with a gamma radiation dose of 25 kGy at 20°C.

Cultures. *Salmonella* Dublin ATCC 15480, *Salmonella* Enteritidis ATCC 13076, *Salmonella* Newport ATCC 6962, *Salmonella* Senftenberg ATCC 8400, and *Salmonella* Typhimurium ATCC 14028 were designated nonvegetable isolates since they have not been associated with an outbreak of disease associated with the ingestion of food sprouts. *Escherichia coli* O157:H7 strains ATCC 35150, ATCC 43889, ATCC 43894, ENT C9490 (associated with a restaurant outbreak and obtained from the Oregon Public Health Laboratory, Portland, Oreg.), and 93-937 (associated with a 1992 outbreak in Oregon and obtained from the Centers for Disease Control [CDC], Atlanta, Ga.) were also designated nonvegetable isolates. The bacterial isolates with ATCC numbers were obtained from the American Type Culture Collection (Manassas, Va.).

Salmonella Anatum F4317, *Salmonella* Infantis F4319, *Salmonella* Newport H1275, and *Salmonella* Stanley H0558 were designated outbreak isolates because they have been associated with outbreaks of salmonellosis associated with the ingestion of sprouts or other vegetables. These isolates were obtained from the CDC. *E. coli* O157:H7 strains F4546, SEA 13B88, and C7929 have been linked to outbreaks associated with the ingestion of sprouts or vegetables and were obtained from the CDC.

All cultures were maintained and cloned on tryptic soy agar (TSA; Difco, Detroit, Mich.). Culture identity was confirmed by Gram staining and on the basis of reactions on the Gram-Negative Identification Cards of the Vitek AMS Automicrobic System (bioMérieux Vitek, Inc., Hazelwood, Mo.) *(2, 13)*. Each isolate was cultured independently in 100 ml of tryptic soy broth (TSB; Difco) at 37°C. Equal amounts of 18-h cultures of each member of a "cocktail" at 37°C in TSB were mixed to prepare the inoculum for each study.

Inoculation of seeds. To inoculate seeds intended for use in studies to determine radiation *D*-values, a 50-ml cocktail of the appropriate organism in TSB was mixed rapidly with 50.0 g of seed by hand massaging the pouch. Immediately after mixing, the excess culture was decanted from the seeds. The bag containing the seeds was placed in a desiccator. The seeds were allowed to dry overnight in vacuo over Drierite (indicating) 97% $CaSO_4$, $CaCl_2$ (W. A. Hammond Drierite Co. Ltd., Xenia, Ohio) at room temperature.

Irradiation. The self-contained gamma radiation source (Lockheed Georgia Company, Marrietta, Ga.) contained 23 ^{137}Cs pencils placed in an annular array around a 63.5-cm-high stainless steel cylindrical chamber with a 22.9-cm internal diameter. The source's strength at the time of the study was ca. 117,355 Ci (4.34 PBq) with a dose rate of 0.10 kGy/min. The dose rate was established with alanine transfer dosimeters from the National Institutes of Standards and Technology, Gaithersburg, Md. Corrections for source decay were made monthly. Routine dosimetry was carried out with 5-mm-diameter alanine dosimeters (Bruker Biospin Corporation, Billerica, Mass.), and the free-radical signal was measured with a Bruker EMS 104 EPR Analyzer *(3)*. Variations in sample dose absorption were minimized by placing small samples within a uniform area of the radiation field, irradiating the samples in a polypropylene container (4-mm wall) to absorb Compton electrons, and using the same geometry for sample irradiation during each study. Under these conditions, the target dose and the actual absorbed dose are the same within the limits of dosimetric measurement, as was confirmed by the routine dosimetric measurements. On the basis of the measurement of dosimeter responses in several experiments, the actual doses were within 2% of the target dose. Samples were maintained at 20 ± 1°C during irradiation through the thermocouple-controlled injection of the gas phase from liquid nitrogen into the top of the irradiation chamber. Sample temperature was monitored continuously with thermocouples that were taped to two samples in the chamber.

Determination of gamma radiation *D*-values. The experimental design for the determination of radiation *D*-values for *Salmonella* and for *E. coli* O157:H7 was as follows: (nonvegetable isolates of *Salmonella*) × (outbreak *Salmonella*) × (2 replications) × (2 seed sources) × (9 radiation doses) = 72 samples. Inoculated seeds were weighed (5.0 g) and placed in the sample section of a polyethylene Stomacher "side filter" no. 400 bag (Tekmar). Each bag was heat sealed after air was expressed from it. The samples were irradiated at 20 ± 1°C to target doses of 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 kGy for *E. coli* O157:H7 and *Salmonella*. Following irradiation, 45 ml of Butterfield's buffer diluent was added to the seeds in each bag, and the mixture was stomached for 90 s. Serial dilutions were prepared, and appropriate dilutions were pour plated in triplicate on TSA. The petri plates were incubated for 24 h at 37°C before the average number of CFU on three petri plates at the dilution producing 30 to 300 colonies was determined. Each study was replicated twice. The average number of CFU per plate for an irradiated sample (N) was divided by the average number of CFU per plate for the untreated control (N_0) to obtain a survival ratio (N/N_0). The *D*-values were the reciprocals of the slopes of the linear regressions of the survivor ratio as determined by least-squares analysis. The zero-dose values were excluded from the calculation of the regression to avoid shoulder effects. Statistical calculations were performed with the general linear model procedure of the SAS statistical package *(9, 19)*. The regressions were tested for differences by analysis of covariance.

Scanning electron microscopy for inoculated seed. Samples of alfalfa seed inoculated with nonvegetable isolates of *Salmonella* were fixed by immersion in 5% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, Pa.) in 0.1 M imidazole ($C_3H_4N_2$-HCl [pH 6.8]; Sigma, St. Louis, Mo.) for 5 min. Seeds

TABLE 1. *Physical characteristics of alfalfa seed and radiation D-values for* Salmonella *on alfalfa seed*[a]

Parameter	Seed source A	Seed source B
Type	Common	Australian
Weight of 1 seed (mg)[b]	1.84 ± 0.18	2.45 ± 0.11
No. of seeds in 1 g	543	408
Bulk density (g/ml)[b]	0.83 ± 0.004	0.83 ± 0.002
Moisture (%)[b]	12.64 ± 2.09	12.39 ± 1.27
a_w[b]	0.52 ± 0.003	0.46 ± 0.001
D for nonvegetable isolates (kGy)[c]	1.02 ± 0.05	0.91 ± 0.06
D for outbreak isolates (kGy)[c]	0.94 ± 0.03	1.02 ± 0.03

[a] Nonvegetable isolates were *Salmonella* Dublin ATCC 15480, *Salmonella* Enteritidis ATCC 13076, *Salmonella* Newport ATCC 6962, *Salmonella* Senftenberg ATCC 8400, and *Salmonella* Typhimurium ATCC 14028. Outbreak isolates were *Salmonella* Anatum F4317, *Salmonella* Infantis F4319, *Salmonella* Newport H1275, and *Salmonella* Stanley H0558.

[b] Mean ± standard deviation.

[c] Mean ± standard error.

were then washed quickly with 0.1 M imidazole and immersed in 50% ethanol to begin the dehydration series. Dehydration was accomplished with 50, 70, 90, and 95% ethanol, each for ca. 1 h. Dehydration was continued with 100% ethanol for seven exchanges.

The samples were then critical-point dried (Denton Vacuum Co., Cherry Hill, N.J.) with liquid CO_2 (BOC) at a medium flow rate for 20 min. Dried samples were mounted on aluminum stubs with colloidal silver paint (EMS) and sputter coated with gold (Edwards Scan Coat 6, Wilmington, Mass.) for 4 min in an argon atmosphere. Samples were observed and digitally imaged on a PGT workstation (Princeton Gamma-Tech, Princeton, N.J.) with a JEOL 840 scanning electron microscope (JEOL, Peabody, Mass.) at an accelerating voltage of 10 kV.

Determination of moisture absorption and drying time for seed. Twenty 10-g samples of alfalfa seed in polyethylene mesh filter bags (Stomacher 400, Tekmar) were each immersed in 10 ml of Butterfield's buffer for 60 s and then drained of excess moisture. The bags were then stored at ambient temperature in vacuo in a vacuum desiccator over 2 lb of anhydrous $CaSO_4$ (W. A. Hammond Drierite Co. Ltd.). Two samples were withdrawn at weekly intervals, and moisture and a_w were determined as described above.

Determination of effect of moisture on *D*-value. Because moisture absorption during inoculation by the seed might alter the radiation *D*-value of a pathogen, a series of studies was conducted to determine whether such effects actually occurred. Radiation-sterilized alfalfa seed was inoculated with the mixture of nonvegetable isolates of *Salmonella*. This study was conducted with the same seed and at the same time as the study involving moisture absorption and drying. The inoculated seed was stored at an ambient temperature in a vacuum desiccator over anhydrous $CaSO_4$. Two samples were withdrawn on day 1 and after 41 days of storage, and the respective radiation inactivation curves were determined for doses of 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 kGy.

RESULTS

The physical and chemical characteristics of seeds influence their penetration by gamma rays and may also affect where and how bacteria may become attached to them. We obtained alfalfa seed from two commercial suppliers and compared their physical characteristics and their radiation *D*-values for *Salmonella*. Probably by chance, the physical characteristics of the seeds from the two sources were significantly different with regard to average weight and water activity (Table 1). These small seeds (543 or 408 seeds weighed 1 g) had a bulk density of 0.83 g/ml (Table 1). Teuber and Brick *(23)* reported a study by Bass in which an average count of 464.5 seeds was found in a study of 418 alfalfa seed lots and 39 cultivars. The alfalfa seed is a typical bean-shaped seed but is quite small, approximately

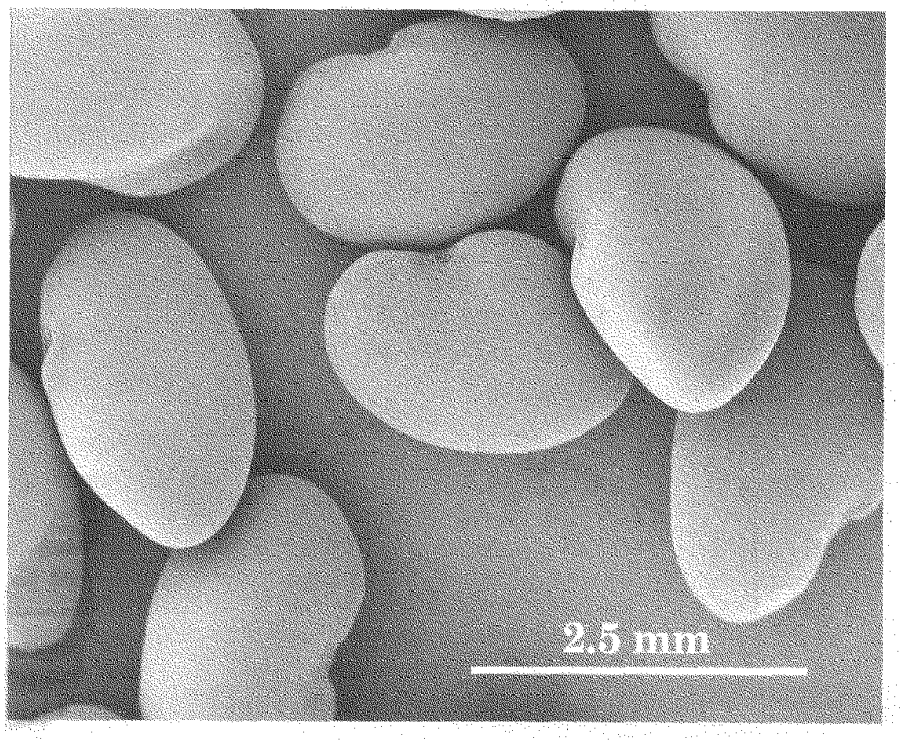

FIGURE 1. *Alfalfa seed inoculated with* E. coli *O157:H7.*

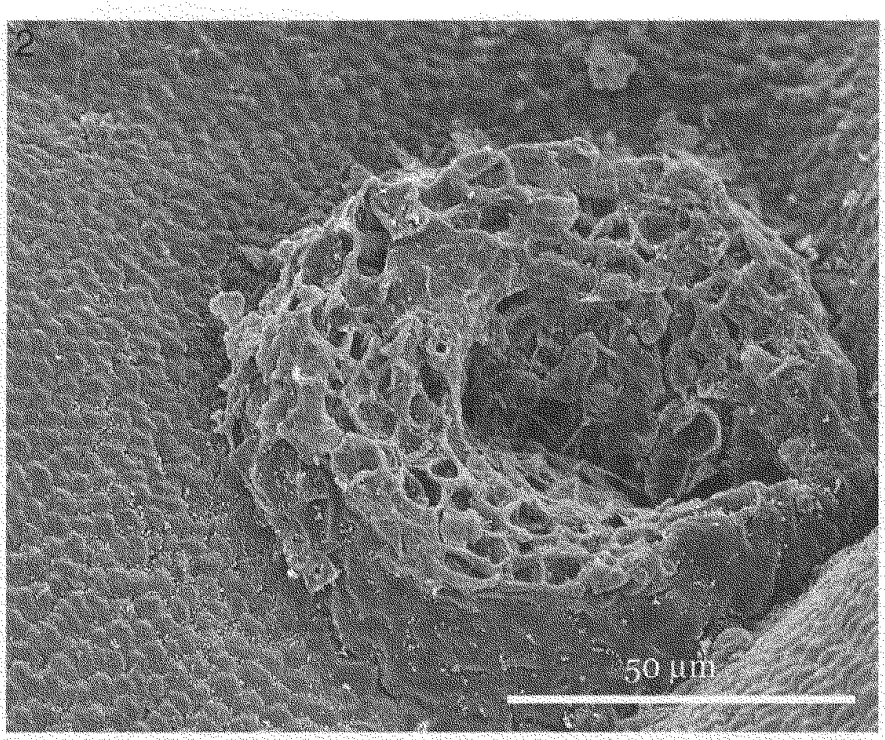

FIGURE 2. *Hilum area of the alfalfa seed inoculated with* E. coli *O157:H7.*

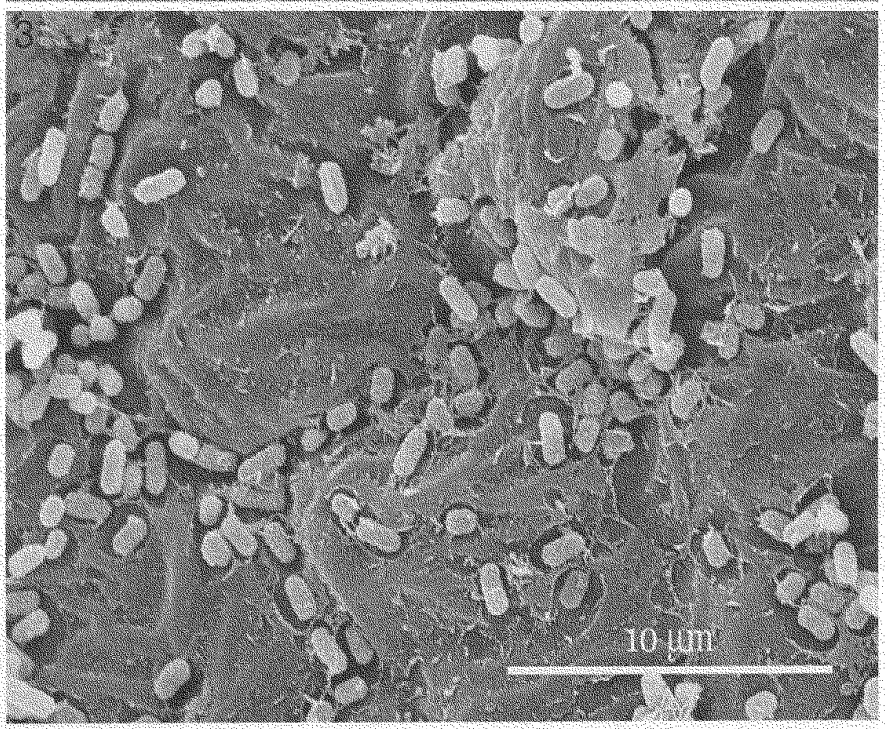

FIGURE 3. *Close-up of a portion of the hilum of the alfalfa seed inoculated with* E. coli *O157:H7.*

1 to 2 mm long, 1 to 2 mm wide, and 1 mm thick *(23)*. The seed from source A weighed significantly less than that from source B, and its a_w value was higher (Table 1). The moisture contents of the seeds were not significantly different, and the mean moisture content for the two (±SD) was 12.52 ± 1.55%.

We needed to answer two questions concerning the process we used to inoculate the seed. First, did our inoculation technique so alter the seed that it would no longer be typical of a "naturally" inoculated seed? If the seed imbibed enough water to result in splitting of the seed coat, it would not be typical of normal seed. Scanning electron micrographs confirmed that the inoculation process did not split the seed coat by initiating germination (Fig. 1); thus, the inoculated seed is believed to be representative of the uninoculated seed. We did not observe a significant number of seeds in which the seed coats had been split. Unfortunately, nobody actually knows what a "naturally inoculated" seed looks like because such seeds make up such a small percentage of the total number of seeds even in a seed lot that is known to be contaminated. Apparently, the very short (1-min) inoculation process we used limited the amount of water that was taken up by the seed, although that amount was nevertheless significant, as described below.

Second, where did the bacterial cells from the inoculum attach to the seeds? *Salmonella* and *E. coli* O157:H7 artificially inoculated onto alfalfa seeds were located pre-

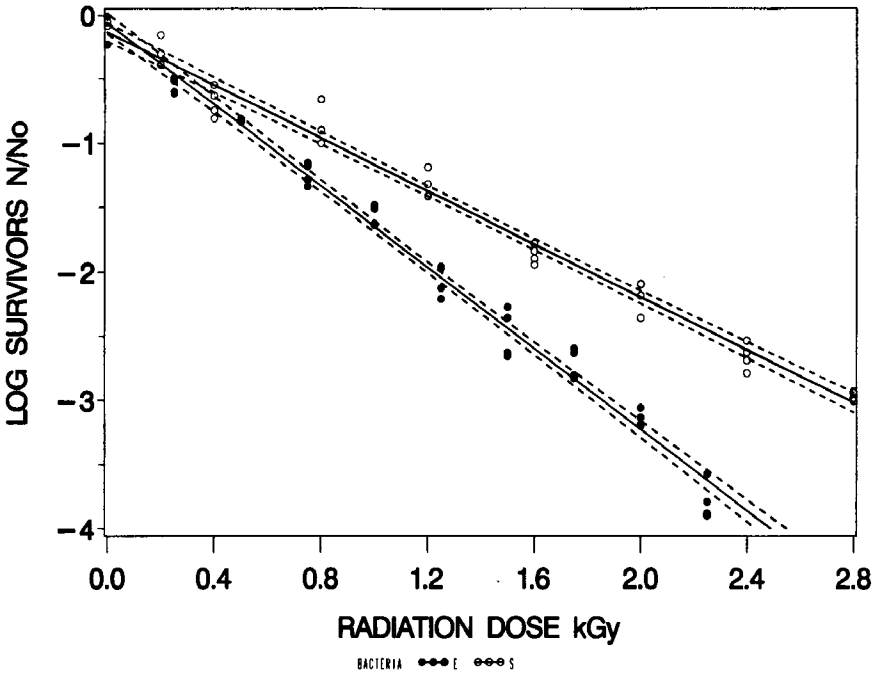

FIGURE 4. *Comparison of the inactivation curves for a mixture of* Salmonella *isolates (*Salmonella *Stanley H0558,* Salmonella *Newport H1275,* Salmonella *Infantis F4319, and* Salmonella *Anatum F4317) (open circles) and a mixture of* E. coli *isolates (*E. coli *F4546, SEA 13B88, and C7929) (dots). The 95% confidence intervals for the estimates of each regression are indicated by the dashed lines.*

dominantly in or on the hilum of the alfalfa seed, as is illustrated for *E. coli* O157:H7 in Figures 2 and 3. Few cells of either *Salmonella* or *E. coli* O157:H7 were observed to be attached at sites other than the hilar region on several dozen seeds examined (Fig. 3). Bacterial cells were observed in the crevices around the conical tips of the palisade cells but primarily in the same hilar region. The hilum, the hilar region, and the papillose seed surface (Fig. 2) appear to provide a very rough area that is ideal for the attachment of the bacterial cells in the inoculum. We can only hypothesize that the cuticle covering the testa (seed coat) *(14)* may have inhibited the attachment of bacteria. The cuticle is composed of cutin and constitutes a complex mixture of fatty acids and waxes *(23).*

The *D*-values for nonvegetable isolates of *Salmonella* for the two seed sources did not differ significantly ($P = 0.01$); the results for the two sources were combined, and a mean *D*-value of 0.96 ± 0.05 kGy was calculated from the inactivation curve (Fig. 4). The *D*-values for the outbreak isolates of *Salmonella* for the two seed sources did not differ significantly ($P = 0.01$); the results for the two seed sources were combined, and a mean *D*-value of 0.98 ± 0.02 kGy was calculated from the inactivation curve. When the regressions for the outbreak and nonvegetable isolates of *Salmonella* were compared by analysis of covariance, they were found to not differ significantly, and a *D*-value of 0.97 ± 0.03 kGy was obtained from the inactivation curve (Fig. 4).

The *D*-value calculated on the basis of the inactivation curves (Fig. 4) for nonvegetable isolates of *E. coli* O157:H7 was 0.55 ± 0.01 kGy, and that for the outbreak strains was 0.60 ± 0.01 kGy. These values are significantly different ($P > F = 0.0034$).

During a preliminary study of the effect of inoculation on the alfalfa seed in which we used buffer in place of an actual inoculum, we observed that the moisture content increased from an initial value of 12.39 to 29.31% and the a_w increased from 0.46 to 0.93 even after drying overnight. The water content of a product is known to affect radiation *D*-values; generally, the greater the water content, the lower the *D*-value *(24).* Thus, we were concerned that *Salmonella* artificially inoculated on seed might have a lower radiation resistance than *Salmonella* on naturally contaminated seed. We used sterile seed from source B to test this conjecture, and we either inoculated the seed as described above or replaced the inoculum with buffer. The *D*-values for nonvegetable isolates of *Salmonella* were determined after inoculation and drying overnight over Drierite and again after the seed had dried back to the original level of moisture. It required approximately 27 to 36 days for the seeds to dry in vacuo over Drierite back to their original moisture content and water activity (Table 1). The initial *D*-value was 1.06 ± 0.04 kGy. The *D*-value for *Salmonella* spp. on the seeds after 36 days of drying in a desiccator was 0.99 ± 0.04 kGy, which was not significantly different from the initial value. The *D*-value calculated from the pooled analyses was 1.02 ± 0.05 kGy. We had expected that the *D*-value might increase as the moisture content decreased. We conclude that either the moisture content of the inoculated seeds did not affect the *D*-value or other properties of the seeds were the determining factor(s) influencing the *D*-values.

The effect of moisture on the nonvegetable isolates of *Salmonella* was further investigated by determining the *D*-value of freeze-dried cells. This value was 0.73 ± 0.07 kGy. We concluded that the high *D*-values observed for the inactivation of *Salmonella* and *E. coli* O157:H7 were due to properties of the seed other than their low moisture content and water activity.

DISCUSSION

Rajkowski and Thayer *(17)* investigated the possibility of inactivating *E. coli* O157:H7 and a cocktail of *Salmonella* isolates on alfalfa sprouts. The results from their study

TABLE 2. *Moisture and water activity of alfalfa seed[a] following simulated inoculation and dessication and the associated* D*-values for* Salmonella[b] *on inoculated and dried seed*

Days of storage	a_w (mean ± SD)	Change in a_w (mean ± SD)	H_2O % (mean ± SD)	Change in H_2O % (mean ± SD)	*D*-value (kGy; mean ± SE)
Initial	0.46 ± 0.01	0	11.78 ± 0.33	0	
0	0.96 ± 0.01	0.50 ± 0.01	23.92 ± 5.79	12.14 ± 5.79	1.06 ± 0.04
7	0.89 ± 0.06	0.43 ± 0.06	22.14 ± 5.90	10.36 ± 5.90	
14	0.79 ± 0.13	0.33 ± 0.13	21.16 ± 0.64	9.38 ± 0.64	
21	0.74 ± 0.07	0.28 ± 0.07	13.78 ± 1.24	2.00 ± 1.24	
27	0.60 ± 0.27	0.14 ± 0.27	15.14 ± 0.86	3.36 ± 0.86	
36	0.50 ± 0.07	0.04 ± 0.07	10.48 ± 0.56	−1.30 ± 0.56	0.99 ± 0.04

[a] Australian seed source B.

[b] *Salmonella* Dublin ATCC 15480, *Salmonella* Enteritidis ATCC 13076, *Salmonella* Newport ATCC 6962, *Salmonella* Senftenberg ATCC 8400, and *Salmonella* Typhimurium ATCC 14028.

indicated that these pathogens could be inactivated by gamma radiation doses very similar to those observed for the same organisms on meat and poultry products. Fan and Thayer *(8)* discovered that the irradiation of alfalfa sprouts at doses typically used for the inactivation of foodborne pathogens did not consistently influence the carotenoid, chlorophyll, and total ascorbic acid contents. The results obtained in this study indicate that the radiation resistance levels of *E. coli* O157:H7 and *Salmonella* are considerably higher when these pathogens are contaminants of alfalfa seed than when they are present on meats or poultry. *Salmonella* on meat and poultry typically have radiation *D*-values of 0.51 to 0.71 kGy, and the *D*-values for *E. coli* O157:H7 on meat and poultry are typically 0.29 to 0.32 kGy *(25)*. The discovery that the radiation dose required to inactivate *Salmonella* on alfalfa seed is approximately 1.0 kGy indicates that the achievement of the 5-log reduction desired by the FDA will require combination treatments, such as one involving a 2-log reduction by irradiation followed by chlorination by the sprout producer. Rajkowski and Thayer *(18)* discovered that the primary effect of a 2-kGy dose on alfalfa seed was a slight decrease in the yield of sprouts. Thus, a dose that would result in a 2-log reduction of *Salmonella* would be acceptable industrially and is permitted by regulations.

The reason(s) for the higher levels of radiation resistance of these foodborne pathogens on the alfalfa seeds than on meat is unknown. Neither the low moisture content nor the water activity of the seed is responsible for the higher levels of resistance to ionizing radiation, because our studies did not find increased radiation resistance as the seeds dried from a moisture content of 12.14% H_2O (a_w = 0.96) to 10.48% H_2O (a_w = 0.50) (Table 2). The location of the bacteria (Fig. 3), at least those associated with artificial inoculation, in the area of the hilum probably provides some protection to the pathogens during washing, but these physical features should not influence gamma radiation sensitivity. These same features might provide some protection to low-energy electrons. We postulate that the decreased sensitivity to ionizing radiation may be due in part to the presence of antioxidants such as ascorbic acid, quercetin, riboflavin, tocopherol, and flavin-3-ols, which are typically associated with seeds, including alfalfa seeds *(4, 7, 10)*. It has been demonstrated that antioxidants can protect foodborne pathogens from radiation *(20, 21)*. The presence of such antioxidants in the seeds does not necessarily indicate that they would be able to interact and protect contaminating bacteria from ionizing radiation, but the potential for them to do so may exist.

The relatively high bulk density of these seeds makes it relatively difficult to obtain a uniform dosage for the product without exceeding the desired 2-kGy maximum dose, posing a problem for the irradiation processor. The ratio of the minimum to the maximum dose could be quite large when full bags of seed are involved. This situation would make the use of vibrating tables and low-energy electron beam devices particularly attractive for this purpose. The use of electrons with an energy level of approximately 300 keV, as proposed by Hayashi et al. *(11)*, seems particularly attractive because such low-energy electrons would penetrate only the surface of the seed and have little effect on the germ of the seed. This technology might allow a 5-log dose to be delivered to the seed to inactivate both *Salmonella* and *E. coli* O157:H7 without significantly affecting the germination ability of the seed or its yield of sprouts.

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